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(54) Title: HAEMOPOIETIC GROWTH FACTOR ANTAGONISTS

(57) Abstract

The present invention relates to modified and variant forms of haemopoietic growth factors (HGF) capable of acting as antagonists to the corresponding native haemopoietic growth factors and their use in ameliorating aberrant effects caused by the native molecules. A modified haemopoietic growth factor (HGF) is characterized by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue. The preferred HGF are granulocyte-macrophage colony-stimulating factor (GM-CSF), interleuking (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, G-CSF and erythropoietin (EPO).

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HAEMOPOIETIC GROWTH FACTOR ANTAGONISTS

The present invention relates to modified and variant forms of haemopoietic growth factors capable of acting as antagonists to the corresponding native haemopoietic growth factors and their use in ameliorating aberrant effects caused by the native molecules.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is one member of a family of haemopoietic growth factors (HGFs) which have a similar predicted tertiary configuration (Parry *et al*, 1988) and whose receptors also belong to a common family (Gearing *et al*, 1989, Bazan, 1990). This family of haemopoietic growth factors includes, for example, in addition to GM-CSF, the cytokines IL-2, IL-3, IL-5, IL-6 and IL-10. A distinct subfamily comprising GM-CSF, IL-3 and IL-5 can be discerned based on structural similarities (Goodall *et al*, 1993) and on their ability to interact with a common receptor component (Lopez *et al*, 1992).

Human GM-CSF (hGM-CSF) comprises 127 amino acids and is available in recombinant form (rhGM-CSF). The hGM-CSF receptor has also been cloned and shown to comprise a binding (α) chain exhibiting low affinity binding to GM-CSF (Gearing *et al*, 1989) and a second (β) chain which does not measurably bind GM-CSF by itself but it allows the formation of a high affinity receptor when co-expressed with the α chain (Hayashide *et al*, 1990).

GM-CSF exhibits a range of activities extending over neutrophil, eosinophil and monocyte lineages. Specifically, GM-CSF stimulates the progenitors of these cells to proliferate and differentiate to become mature cells. In addition, it stimulates mature cells to greater function. The stimulation of mature cells results in greater capacity to phagocytose and kill micro-organisms, kill antibody-coated tumour cells and parasites and generate superoxide anion (O_2^-) in response to various stimuli. The purpose of this activation is presumed to enable the mature cells to become better effector cells in inflammatory reactions.

Therapeutically, the HGFs form an important group of molecules for their actual or potential properties. For example, the main indications for GM-CSF are for its effects on progenitor cells or mature cells. Using its effects on progenitor cells, GM-CSF is used in the treatment of bone marrow failure as seen in aplastic anaemia or chemotherapy or AIDS-induced marrow suppression. In the treatment of infections, the capacity to stimulate mature cells is especially relevant. The capacity of GM-CSF-activated neutrophils and eosinophils to kill tumour cells that have bound antibody is especially remarkable and could be used in tumour therapy.

However, despite the actual and potential benefits of HGFs, they can exhibit some detrimental side effects. For example, GM-CSF can exhibit toxicity due to stimulation of mature cells causing blood vessel damage or thrombosis. The eosinophilia caused by GM-CSF appears especially damaging in this regard. The molecule can also have detrimental effects by stimulating growth of leukaemia cells and tumour cells of non-haemopoietic origin and stimulating production of inflammatory mediators.

International Patent Application No. PCT/AU89/00177 and an article by Lopez *et al.* (1992) disclose amino acid variants of GM-CSF which have exhibited reduced potency. These variants were investigated further for their potential as GM-CSF antagonists. However, the variants cause classical stimulation at concentrations 100 fold greater compared to the native GM-CSF molecule. Furthermore, attempts to find antagonistic properties failed since mixing large concentrations of one of the variants with suboptimal concentrations of native GM-CSF resulted in stronger GM-CSF stimulation with no

evidence of inhibition being observed.

There is a need, therefore, to develop antagonists to HGFs and in particular GM-CSF which are capable of ameliorating the aberrant effects of the corresponding native molecules. There is also a need for such antagonists not to exhibit agonist properties in respect of the corresponding HGFs.

Accordingly, one aspect of the present invention provides a haemopoietic growth factor characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

In accordance with the present invention, it is proposed that the modified HGFs defined above act as antagonists of the native form of the corresponding HGF but not other HGFs. The term "modified" is considered herein synonymous with terms such as "variant", "derivative" and "mutant".

The HGFs are preferably GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) modified in accordance with the present invention. Most preferably, the HGF is GM-CSF. The HGFs are preferably in recombinant or synthetic form and, with the exception of the amino acid substitution(s) in the first α-helix, the amino acid sequence of the HGF may be the same as the naturally occurring molecule (i.e. native molecule) or may carry single or multiple amino acid substitutions, deletions and/or additions to the native amino acid sequence. The HGF sequences are preferably of mammalian origin such as from humans, livestock animals, companion animals or laboratory test animals. Most preferably, the HGFs are of human origin or of a mammalian origin capable of functioning in humans.

The first α-helix of GM-CSF has been determined at 2.4 angstrom resolution by X-ray crystallography and encompasses amino acid residues 13 to 28. Similar procedures may be adopted to determine the first α-helix in other haemopoietic growth factors. The position may also be determined by analogy to GM-CSF structure.

Reference to "unglycosylated form" herein means that the molecule is completely unglycosylated such as when expressed in recombinant form in a prokaryotic organism (e.g. *E. coli*). Alternatively, a glycosylation-deficient mammalian cell may be used or complete deglycosylation may occur *in vitro* using appropriate enzymes. Accordingly, the present invention extends to chemically synthesised GM-CSF which is in unglycosylated form.

An "exposed" amino acid is taken herein to refer to an amino acid on an exposed or outer portion of an α-helix compared to those amino acids orientated towards the inside of the molecule.

An acidic amino acid includes, for example, Glu and Asp. Preferred basic amino acids are Arg and Lys.

According to another aspect of the present invention, there is provided a haemopoietic growth factor characterised by:

(i) being in unglycosylated form;

(ii) comprising a sequence of amino acids within the first α-helix;

(iii) one or more exposed amino acids in said α-helix which have acidic or acidic-like properties being substituted by a basic amino acid residue;

(iv) being in recombinant or synthetic form;

(v) being capable of acting as an antagonist for at least one property of the corresponding native HGF.

This aspect of the present invention is predicated in part on the surprising discovery that a mutation in amino acid 21 (Glu) of hGM-CSF to Arg or Lys together with the variant in GM-CSF being in unglycosylated form results in the hGM-CSF variant being unable to detectably exhibit classical GM-CSF function. The variants, referred to herein "GM-CSF Arg^{21}" and "GM-CSF Lys^{21}" are unable to bind to high affinity receptors but are still able to fully bind the low affinity α chain of the GM-CSF receptor. Importantly, the non-glycosylated GM-CSF Arg^{21} and GM-CSF Lys^{21} act as antagonists, preventing the stimulatory effect of native GM-CSF. For convenience, the numbering of amino acid

residues in hGM-CSF is taken from Wong *et al.* (1985).

By way of a shorthand notation the following three letter abbreviations for amino acid residues are used in the specification as defined in Table 1.

Where a specific amino residue is referred to by its position in the polypeptide of an HGF, the amino acid abbreviation is used with the residue number given in superscript (i.e. Xaa^n, wherein Xaa is the amino acid residue).

Table 1

Amino acid	Three-letter abbreviation	Corresponding single-letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The present invention is exemplified using GM-CSF and in particular hGM-CSF Arg^{21} and hGM-CSF Lys^{21}. This is done, however, with the understanding that the present invention extends to all HGFs as hereinbefore described.

For example, given that there is a Glu at position 22 of IL-3 and position 13 in IL-5 which in the three dimensional structure (in the case of IL-5) occupies an equivalent position to Glu^{21} of GM-CSF, then the present invention provides the basis for the creation of antagonists in IL-3 and IL-5. The substitution of Glu at these positions in IL-3 and IL-5 may be the sole mutation or it may be in combination with other amino acid mutations (including substitutions, deletions and/or additions) for the development of effective antagonists. This similarly applies to other HGFs based on the acidic or acidic-like amino acid residues in the first α-helix of the molecule. The location of the N-terminal helix can in each case be readily determined on comparable motifs from predicted helices. Such HGFs are listed in Table 2 showing the acidic or acidic-like amino acid residue in bold in the equivalent position to Glu^{21} of hGM-CSF. The acidic or acidic-like amino acid residues are readily substituted by, for example, recombinant DNA technology.

According to another aspect of the present invention there is provided a modified variant including HGF comprising an amino acid sequence in the first α-helix of said HGF selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu (SEQ ID No. 1);
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID No. 2);
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu (SEQ ID No. 3);
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu (SEQ ID No. 4);
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu (SEQ ID No. 5);
vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys (SEQ ID No. 6);
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile (SEQ ID No. 7);

viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID No. 8);

ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser (SEQ ID No. 9);

x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile (SEQ ID No. 10); and

xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly (SEQ ID No. 11);

wherein Xaa is a basic amino acid, preferably selected from the group consisting of Arg and Lys, and wherein said variant HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF. Preferably, the haemopoietic growth factor is hGM-CSF and Xaa is Arg or Lys at position 21 of the first α-helix.

The HGF antagonists of the present invention and in particular GM-CSF Arg^{21} and GM-CSF Lys^{21} are useful *inter alia* in the treatment of myeloid and lymphocyte leukaemias, some tumours of non-haemopoietic origins and acute and chronic inflammation such as asthma and rheumatoid arthritis. These and other conditions are considered herein to result from or be facilitated by the aberrant effects of an endogenous HGF such as GM-CSF. hGM-CSF Arg^{21} and hGM-CSF Lys^{21} will also be useful in mobilising stem cells and progenitor cells into the circulation without the risk of activating neutrophils and monocytes. Other related molecules may have different useful properties.

The present invention, therefore, contemplates a method of treatment comprising the administration to a mammal of an effective amount of a modified HGF as hereinbefore defined and in particular hGM-CSF Arg^{21} or hGM-CSF Lys^{21} or both for a time and under conditions sufficient for effecting said treatment. Generally, the mammal is a human, livestock animal, companion animal or laboratory test animal. Most preferably, the mammal is a human. A single modified HGF may be administered or a combination of variants of the same HGF. For example, a range of hGM-CSF variants could be used such as a combination of hGM-CSF Arg^{21} and hGM-CSF Lys^{21}.

TABLE 2

Cytokines related to GM-CSF exhibit a conserved acidic residue analogous to E21 in GM-CSF

CYTOKINE[2]	HELIX[1] (Amino Acid Residue No.)	AMINO ACID SEQUENCE	SEQ ID No.
hGM-CSF	15-28	His Val Asn Ala Ile Gln **Glu** Ala Arg Arg Leu Leu Asn Leu	12
hIL-5	9-24	Ala Leu Val Lys **Glu** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu	13
hIL-3	18-27	Asn Met Ile **Asp** Glu Ile Ile Thr His Leu	14
hIL-2	17-25	Leu Leu Leu **Asp** Leu Gln Met Ile Leu	15
hIL-4	8-17	Ile Thr Leu Gln **Asp** Ile Ile Lys Thr Leu	16
hIL-6	18-43	Arg Tyr Ile Leu **Asp** Gly Ile Ser Ala Leu Arg Lys	17
hIL-7	9-20	Gly Asp Gln Tyr **Glu** Ser Val Leu Met Val Ser Ile	18
hIL-9	7-22	Ala Gly Ile Leu **Asp** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp	19
hIL-10	21-31	Asn Met Leu Arg **Asp** Leu Arg Asp Ala Phe Ser	20
hG-CSF	13-24	Phe Leu Leu Lys Cys Leu **Glu** Gln Val Arg Lys Ile	21
hEPO	4-28	Tyr Leu Leu Glu Ala Lys **Glu** Ala Glu Asn Ile Thr Thr Gly	22

1 Only the pertinent portion of each helix is shown.

2 Predicted helices are from: for IL-2, (Brandhuber *et al*, 1987; Zurawski and Zurawski, 1989); for hIL-3 (Parry *et al*, 1988); for mIL-5, (Parry *et al*, 1988); for hIL-6 (Bazan, 1990a); for hG-CSF, (Parry *et al*, 1988); for hEPO, (Bazan, 1990); for hGM-CSF the first helix was determined from the crystal structure (Karplus, 1991). The location of the N-terminal helix in the other cytokines was based on comparable motifs from these secondary structure predictions.

The present invention also provides a pharmaceutical composition comprising the variant HGFs as hereinbefore defined or combinations thereof. Most particularly, the pharmaceutical composition comprises hGM-CSF Arg^{21} or hGM-CSF Lys^{21} or both.

Methods for preparing pharmaceutical compositions are well known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, Mack Publishing Company, Eaton, Pennsylvania, USA and may also include one or more pharmaceutical acceptable carriers and/or diluents.

The present invention is further described by reference to the following non-limiting Examples and/or Figures.

In the Figures:

Figure 1 is a graphical representation showing titration of *E. coli* derived GM-CSF Arg^{21} for its ability to affect 0_2^- production in human neutrophils (□) and to antagonise the enhancement of 0_2^- by wild type GM-CSF tested at 1.0 ng/ml (△) and at 0.1 ng/ml (closed triangle).

Figure 2 is a graphical representation showing failure of *E. coli*-derived GM-CSF Arg^{21} to antagonise the enhancement of 0_2^- production in human neutrophils stimulated with tumour necrosis factor-α (TNF-α).

Figure 3 is a graphical representation showing competitive inhibition of ^{125}I-GM-CSF binding to COS cells transfected with the GM-CSF receptor α chain alone (top) or α and β chains (bottom) by GM-CSF Arg^{21}.

Figure 4 is a graphical representation showing titration of GM-CSF Arg^{21} for its ability to antagonise GM-CSF (A) in contrast to no effect on IL-3 (B)-mediated proliferation of TF-1 cells.

Figure 5 is a graphical representation showing the titration of GM-CSF Arg^{21} for its ability to antagonise TF-1 proliferation stimulated by either *E. coli*-derived GM-CSF (A), yeast-derived GM-CSF (B) or CHO-derived GM-CSF (C).

Figure 6 is a graphical representation showing the titration of GM-CSF Arg^{21} for its ability to antagonise three primary human myeloid leukaemia (A, B & C) *ex vivo*.

Figure 7 is a graphical representation showing that E21R antagonises both GM-CSF but not TNF-α-mediated stimulation of human neutrophils (A), and both E21R and E21K also antagonise neurtrophil stimulation by CHO cell-derived GM-CSF (B). In panel A, titrations of *E. coli*-derived wild type GM-CSF (●), TNF-α (◆) and E21R (■) are shown. In antagonistic experiments, E21R was titrated against 1ng/ml of *E. coli*-derived GM-CSF (□) or 3ng/ml TNF-α (◊). In panel B, titrations of CHO cell-derived wild type GM-CSF (○), E21R (■) and E21K (▲) are shown. In antagonistic experiments, E21R (□) or E21K (△) were titrated against 3ng/ml CHO cell-derived GM-CSF. Each value represents the mean of triplicate determinations and error bars represent the SEM.

EXAMPLE 1

Expression of wild type and GM-CSF Arg^{21} and GM-CSF Lys^{21} in an *E. coli* expressed system

Wild type GM-CSF was expressed in *E. coli* using a plasmid (designated pshGM-CSF) containing a synthetic human GM-CSF cDNA cloned into the *E. coli* expression vector pIN-III-OmpH3, a derivative of the vector pIN-III-OmpA2 (Ghrayeb *et al*, 1984). GME21R was expressed from the plasmid pSGM21.1 containing $Glu^{21} \rightarrow Arg^{21}$ substitution and was derived from the pSGM-CSF parental plasmid. GME21K was expressed from the plasmid pSGM21.4 containing $Glu^{21} \rightarrow Lys^{21}$ substitution and was derived from the pSGM-CSF parental plasmid.

pSGM21 was generated by initially eliminating a SacII site from the wild type GM-CSF using oligonucleotide cassette mutagenesis to generate plasmid pSGMV1. A 64 bp Nco 1/SacII fragment was then excised from the pSGMV1 plasmid and replaced by double-stranded 64bp oligonucleotides containing the appropriate mutation in the DNA sequence.

pSGM21.4 was generated by excising an 88 bp Bgl11/SacII fragment from pSGF1 and replacing it with 88bp oligonucleotides containing the appropriate mutation site directed mutagenesis (Zoller & Smith, 1984).

Protein was expressed in either MC1061, for wild type GM-CSF or BL21 for GME21R or GME21K, after induction by isopropyl β-D-thiogalactoside and recovered from the periplasmic space by osmotic shock (Koshland and Botstein, 1980).

GM-CSF protein was purified using a monoclonal antibody 4A12 generated in the laboratory coupled to Sepharose beads. Further purification was achieved by reversed phase HPLC using a BioRad controller and a Brownlee Aquaport C8 100 x 10mm column. GM-CSF was eluted using a 30-50% gradient of acetonitrile in 0.1% trifluoroacetic acid.

Resultant purified GM-CSF was lyophilised and resuspended in 1 x PBS before being quantitated by HPLC gel filtration. Samples were fractionated on a Beckman Ultraspherogel SEC3000 7.5 x 300mm using a 0.1M Na Phosphate pH 7.0/01M Na_2SO_4 mobile phase. Purity was estimated at >95% and area under peaks corresponding to GM-CSF integrated as the extinction coefficient of 0.95 absorbance units.ml.mg^{-1}.

EXAMPLE 2

Visualisation of mutant GM-CSF protein

GM-CSF unpurified or purified from *E. coli* was size-fractionated by $NaDodSo_4$/12.5% w/v polycarylamide gel electrophoresis (Laemmli, 1970). For Western blot analysis, protein was transferred to nitrocellulose as described (Towbin *et al,* 1979). Filters were probed with a sheep anti-GM-CSF followed by a second layer of biotinylated-rabbit anti-sheep IgG. After a further incubation with an avidin-biotinylated-horseradish peroxidase conjugate, the complex was visualised using a diaminobenzidine substrate solution. For silver staining, the method of Morrissey (1981) was used.

EXAMPLE 3

Stimulation of haemopoietic cell proliferation

The human erythroleukaemia cell line TF-1 (and myeloid leukaemia) cells were used to measure the proliferative function of GM-CSF and GM-CSF Arg^{21}. Proliferation of TF-1 cells were measured by the ability to incorporate [^{3}H]-thymidine in response to increasing doses of GM-CSF. This assay was performed as described by Lopez *et al* (1988).

EXAMPLE 4

Functional activation of human granulocytes and monocytes

The superoxide anion production assay was carried out as previously described (Lopez *et al,* 1986).

EXAMPLE 5

Radioreceptor assay

(a) Radioiodination of GM-CSF.

Yeast derived human GM-CSF or *E. coli*-derived human GM-CSF was radioiodinated by the ICl method (Contreras *et al*, 1983). Iodinated protein was separated from free ^{125}I by chromatography on a Sephadex G-25 PD10 column (Pharmacia, Uppsala, Sweden), equilibrated in phosphate buffered saline (PBS) containing 0.02% w/v Tween 20, and stored at 4°C for up to 4 weeks. Before use, the iodinated protein was purified from Tween and non-protein-associated radioactivity by cation exchange chromatography on a 0.3ml CM-Sepharose CL-6B column (Pharmacia) and stored at 4°C for up to 5 days. The radiolabelled GM-CSF retained >90% biological activity as judged from titration curves using non-iodinated GM-CSF as controls.

(b) Competition binding assays.

Competition for binding to high affinity and low affinity receptors used stably transfected CHO cell lines expressing either the α and β chains, or the α chain alone. The cells were suspended in binding medium consisting of RPMI-1640 supplemented with 20mmol/l HEPES and 0.5% w/v bovine serum albumin (BSA) and 0.1% w/v sodium azide. Typically, equal volumes (50μl) of 4 x 10^4 CHO cells, iodinated GM-CSF and different

concentrations of GM-CSF and GM-CSF Arg^{21} were mixed in siliconised glass tubes for 3 hr at 4°C. At the end of the incubation period, cell suspensions were overlaid on 0.2ml foetal calf serum (FCS) at 4°C, centrifuged in a Beckman Microfuge 12, and the tip of each tube containing the visible cell pellet cut off and counted in a gamma counter. Specific counts were determined by first subtracting the counts, obtained in the presence of excess wild type GM-CSF.

EXAMPLE 6

Generation of hGM-CSF Variants

By way of example only, the generation of GM-CSF Arg^{21} is hereinafter described in detail. A human GM-CSF cDNA was subjected to mutagenesis to introduce the amino acid Arg for Glu at position 21. Two mutants were obtained, one containing the $Glu^{21}\rightarrow$Arg mutation and a second one containing a double mutation $X^{10}\rightarrow$Ile and $Glu^{21}\rightarrow$Arg. These mutants were cloned into the expression system piN OMPIII and expressed in *E. coli*. Wild type (WT) GM-CSF was expressed in MC1061. GM-CSF Arg^{21} could not be expressed in MC1061. Of twenty strains tested for GM-CSF Arg^{21} expression, BL21 was the highest producer and used for subsequent studies.

To obtain purified GM-CSF Arg^{21} in high yields a two-step purification procedure was devised. In the first step, GM-CSF Arg^{21} was purified by an affinity column constructed with a monoclonal antibody (4A12) that binds to GM-CSF in solution and with high affinity. Affinity-purified GM-CSF Arg^{21} was then purified by reverse-phase HPLC and quantitated by HPLC before being analysed for biological and binding activities.

It was found that *E. coli*-derived GM-CSF Arg^{21} was unable to enhance neutrophil 0_2-production up to a concentration of 3,000 ng/ml (Figure 1). This is different to the inventors' previous results with CHO-derived (i.e. glycosylated) GM-CSF Arg^{21} which was able to enhance neutrophil function at approximately 30 ng/ml which represents a 300 fold reduced potency compared to wild type GM-CSF (Lopez *et al*, 1992).

Despite its inability to activate neutrophils, *E. coli*-derived GM-CSF Arg^{21} was able to bind as well as wild type GM-CSF to the α-chain of the GM-CSF receptor (Figure 3). In contrast, GM-CSF Arg^{21} exhibited an approximate 100-fold reduction in binding to the αβ GM-CSF receptor complex (Figure 3) indicating that the influence of the β chain has been selectively lost.

This is the first time that a GM-CSF mutant is shown to have unaltered binding to the GM-CSF receptor α-chain as well as being devoid of agonistic activity. This indicates that whilst binding to the α chain is necessary for GM-CSF activity it is not sufficient, and that GM-CSF binding to the β chain is required for GM-CSF-mediated activation.

Since *E. coli*-derived GM-CSF Arg^{21} was not able to stimulate neutrophils yet fully bound the GM-CSF receptor α chain, it was tested for antagonistic activity. The inventors found that this mutant fully inhibited the effect of wild type GM-CSF with an approximately 300-fold excess required to induce 50% inhibition of *E. coli*-derived wild type GM-CSF (Figure 1). This antagonistic effect was specific for GM-CSF as judged by the lack of antagonistic effect of GM-CSF Arg^{21} on TNF enhancement of neutrophil 0_2^- production (Figure 2).

The antagonistic effect of *E. coli*-derived GM-CSF Arg^{21} was present whether wild type GM-CSF was expressed in *E. coli* (Figure 5A), yeast (Figure 5B) or CHO cells (Figure 5C). In keeping with the differences in binding affinity between heavily glycosylated CHO GM-CSF (lower affinity), partially glycosylated yeast GM-CSF (intermediate affinity) and unglycosylated *E. coli* GM-CSF (higher affinity), *E. coli*-derived GM-CSF Arg^{21} antagonised better the CHO wild type GM-CSF (Figure 5).

The antagonism of *E. coli*-derived GM-CSF Arg^{21} was not restricted to proliferation of the established TF-1 cell line but was also seen in primary myeloid leukaemias. In three different leukaemias, *E. coli*-derived GM-CSF Arg^{21} antagonised the proliferative effect of wild type *E. coli* GM-CSF with an EC50 that varied with each leukaemia (Figure 6).

These results show that an unglycosylated GM-CSF molecule with a mutated Glu for an Arg in position 21 of the first α-helix is able to antagonise native GM-CSF.

Since the mutated Glu in GM-CSF is in a position where the same acidic residue or similar acidic residues (e.g. Asp) are present in related growth factors (see Table 2), this invention extends to antagonistic molecules for these growth factors constructed by incorporating the analogous charge reversal mutation. In particular, given that the GM-CSF, IL-3 and IL-5 receptor share the β chain, the Glu^{22} in IL-3 and the Glu^{13} in IL-5 are predicted to play a similar role. Other variant HGFs are shown in Examples 3 to 22 in which the equivalent or similar amino acid residue to Glu^{21} of hGM-CSF is replaced by either Arg or Lys. In these Examples, the amino acid sequences are provided for the relevant portion of the first α-helix carrying the substitution (see Table 2).

EXAMPLE 7

hGM-CSF Lys^{21}

His Val Asn Ala Ile Gln **Lys** Ala Arg Arg Leu Leu Asn Leu (SEQ ID NO. 23)

EXAMPLE 8

hIL-5 Lys^{13}

Ala Leu Val Lys **Lys** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID NO.24)

EXAMPLE 9

hIL-5 Arg^{13}

Ala Leu Val Lys **Arg** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID NO. 25)

EXAMPLE 10

hIL-3 Variants

Asn Met Ile **Asp Lys** Ile Ile Thr His Leu	hIL-3 Lys^{22}	(SEQ ID NO. 26)
Asn Met Ile **Lys Glu** Ile Ile Thr His Leu	hIL-3 Lys^{21}	(SEQ ID NO. 27)
Asn Met Ile **Asp Arg** Ile Ile Thr His Leu	hIL-3 Arg^{22}	(SEQ ID NO. 28)
Asn Met Ile **Arg Glu** Ile Ile Thr His Leu	hIL-3 Arg^{21}	(SEQ ID NO. 29)
Asn Met Ile **Lys Lys** Ile Ile Thr His Leu	hIL-3 Lys^{21} Lys^{22}	(SEQ ID NO. 30)

Asn Met Ile **Arg Arg** Ile Ile Thr His Leu hIL-3 Arg^{21} Arg^{22} (SEQ ID NO. 31)

EXAMPLE 11

hIL-2 Lys^{20}

Leu Leu Leu **Lys** Leu Gln Met Ile Leu (SEQ ID NO. 32)

EXAMPLE 12

hIL-2 Arg^{20}

Leu Leu Leu **Arg** Leu Gln Met Ile Leu (SEQ ID NO. 33)

EXAMPLE 13

hIL-4 Lys^{12}

Ile Thr Leu Gln **Lys** Ile Ile Lys Thr Leu (SEQ ID NO. 34)

EXAMPLE 14

hIL-4 Arg^{12}

Ile Thr Leu Gln **Arg** Ile Ile Lys Thr Leu (SEQ ID NO. 35)

EXAMPLE 15

hIL-6 Lys^{22}

Arg Tyr Ile Leu **Lys** Gly Ile Ser Ala Leu Arg Lys (SEQ ID NO. 36)

EXAMPLE 16

hIL-6 Arg^{22}

Arg Tyr Ile Leu **Arg** Gly Ile Ser Ala Leu Arg Lys (SEQ ID NO. 37)

EXAMPLE 17

hIL-7 Lys^{13}

Gly Asp Gln Tyr **Lys** Ser Val Leu Met Val Ser Ile (SEQ ID NO. 38)

EXAMPLE 18

hIL-7 Arg^{13}

Gly Asp Gln Tyr **Arg** Ser Val Leu Met Val Ser Ile (SEQ ID NO. 39)

EXAMPLE 19

hIL-9 Lys^{11}

Ala Gly Ile Leu **Lys** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID NO. 40)

EXAMPLE 20

hIL-9 Arg^{11}

Ala Gly Ile Leu **Arg** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID NO. 41)

EXAMPLE 21

hIL-10 Lys^{25}

Asn Met Leu Arg **Lys** Leu Arg Asp Ala Phe Ser (SEQ ID NO. 42)

EXAMPLE 22

hIL-10 Arg^{25}

Asn Met Leu Arg **Arg** Leu Arg Asp Ala Phe Ser (SEQ ID NO. 43)

EXAMPLE 23

hG-CSF Lys^{19}

Phe Leu Leu Lys Cys Leu **Lys** Gln Val Arg Lys Ile (SEQ ID NO. 44)

EXAMPLE 24

hG-CSF Arg^{19}

Phe Leu Leu Lys Cys Leu **Arg** Gln Val Arg Lys Ile (SEQ ID NO. 45)

EXAMPLE 25

hEPO Lys^{10}

Tyr Leu Leu Glu Ala Lys **Lys** Ala Glu Asn Ile Thr Thr Gly (SEQ ID NO. 46)

EXAMPLE 26

hEPO Arg^{10}

Tyr Leu Leu Glu Ala Lys **Arg** Ala Glu Asn Ile Thr Thr Gly (SEQ ID NO. 47)

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

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(C) OPERATING SYSTEM: PC-DOS/MS-DOS
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(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acid residues
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

Asn Met Ile Xaa Xaa Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

Leu Leu Leu Xaa Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 7:

Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 8:

Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 9:

Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 10:

Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 11:

Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID NO. 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 12:

His Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 13:

Ala Leu Val Lys Glu Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

Asn Met Ile Asp Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

Leu Leu Leu Asp Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 16:

Ile Thr Leu Gln Asp Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 17:

Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 18:

Gly Asp Gln Tyr Glu Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 19:

Ala Gly Ile Leu Asp Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 20:

Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 21:

Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 22:

Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID NO. 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 23:

His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 24:

Ala Leu Val Lys Lys Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 25:

Ala Leu Val Lys Arg Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 26:

Asn Met Ile Asp Lys Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 27:

Asn Met Ile Lys Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 28:

Asn Met Ile Asp Arg Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 29:

Asn Met Ile Arg Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 30:

Asn Met Ile Lys Lys Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 31:

Asn Met Ile Arg Arg Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 32:

Leu Leu Leu Lys Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 33:

Leu Leu Leu Arg Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 34:

Ile Thr Leu Gln Lys Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 35:

Ile Thr Leu Gln Arg Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 36:

Arg Tyr Ile Leu Lys Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 37:

Arg Tyr Ile Leu Arg Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 38:

Gly Asp Gln Tyr Lys Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 39:

Gly Asp Gln Tyr Arg Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 40:

Ala Gly Ile Leu Lys Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 41:

Ala Gly Ile Leu Arg Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 42:

Asn Met Leu Arg Lys Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 43:

Asn Met Leu Arg Arg Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 44:

Phe Leu Leu Lys Cys Leu Lys Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 45:

Phe Leu Leu Lys Cys Leu Arg Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 46:

Tyr Leu Leu Glu Ala Lys Lys Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID. NO. 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 47:

Tyr Leu Leu Glu Ala Lys Arg Ala Glu Asn Ile Thr Thr Gly

CLAIMS:

1. A modified haemopoietic growth factor (HGF) characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

2. A modified HGF according to claim 1, wherein said HGF is a modified form of an HGF selected from granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL) -2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, erythropoietin (EPO).

3. A modified HGF according to claim 2 wherein said HGF is a modified form of GM-CSF.

4. A modified HGF according to claim 1 or 2 or 3 wherein said HGF is of human, livestock animal, companion animal or laboratory test animal origin.

5. A modified HGF according to claim 4 wherein said HGF is of human origin.

6. A modified HGF according to any one of the preceding claims wherein the acidic amino acid residue on the first α-helix is Glu and/or Asp and the basic amino acid residue substituted therefor is Arg and/or Lys.

7. A modified HGF comprising an amino acid sequence in the first α-helix of said HGF selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu;
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu;
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu;
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu;
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu;

vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys;
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile;
viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp;
ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser;
x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile;
xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly;

wherein Xaa is a basic amino acid, selected from the group consisting of Arg and Lys and wherein said modified HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF.

8. A modified HGF according to claim 7 wherein the HGF is a modified form of an HGF selected from the list consisting of GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, EPO.

9. A modified HGF according to claim 8, wherein the variant HGF is a modified form of GM-CSF.

10. A modified HGF according to claim 7 or 8 wherein Xaa is Arg.

11. A modified human GM-CSF in unglycosylated form having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Arg Ala Arg Arg Leu Leu Asn Leu.

12. A modified human GM-CSF in unglycosylated form having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu.

13. A modified human IL-5 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Leu Val Lys Lys Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu.

14. A modified human IL-5 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Leu Val Lys Arg Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu.

15. A modified human IL-3 in unglycosylated form having an amino acid sequence in a first α-helix selected from the list consisting of:

Asn Met Ile Asp Lys Ile Ile Thr His Leu;
Asn Met Ile Lys Glu Ile Ile Thr His Leu;
Asn Met Ile Asp Arg Ile Ile Thr His Leu;
Asn Met Ile Arg Glu Ile Ile Thr His Leu;
Asn Met Ile Lys Lys Ile Ile Thr His Leu; and
Asn Met Ile Arg Arg Ile Ile Thr His Leu.

16. A modified human IL-2 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Leu Leu Leu Lys Leu Gln Met Ile Leu.

17. A modified human IL-2 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Leu Leu Leu Arg Leu Gln Met Ile Leu.

18. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ile Thr Leu Gln Lys Ile Ile Lys Thr Leu.

19. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ile Thr Leu Gln Arg Ile Ile Lys Thr Leu.

20. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Arg Tyr Ile Leu Lys Gly Ile Ser Ala Leu Arg Lys.

21. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Arg Tyr Ile Leu Arg Gly Ile Ser Ala Leu Arg Lys.

22. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Gly Asp Gln Tyr Lys Ser Val Leu Met Val Ser Ile.

23. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Gly Asp Gln Tyr Arg Ser Val Leu Met Val Ser Ile.

24. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Gly Ile Leu Lys Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp.

25. A modified human IL-4 having an amino acid sequence in a first α-helix comprising
Ala Gly Ile Leu Arg Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp.

26. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Asn Met Leu Arg Lys Leu Arg Asp Ala Phe Ser.

27. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Asn Met Leu Arg Arg Leu Arg Asp Ala Phe Ser.

28. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Phe Leu Leu Lys Cys Leu Lys Gln Val Arg Lys Ile.

29. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Phe Leu Leu Lys Cys Leu Arg Gln Val Arg Lys Ile.

30. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Tyr Leu Leu Glu Ala Lys Lys Ala Glu Asn Ile Thr Thr Gly.

31. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Tyr Leu Leu Glu Ala Lys Arg Ala Glu Asn Ile Thr Thr Gly.

32. A method of ameliorating the aberrant effects of an endogenous HGF in a mammal, said method comprising administering to said mammal an effective amount of a modified HGF characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-heliz wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

33. A method according to claim 32 wherein the modified HGF is selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu;
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu;
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu;
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu;
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu;
vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys;
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile;

viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp;

ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser;

x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile; and

xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly;

wherein Xaa is a basic amino acid selected from the group consisting of Arg and Lys and wherein said variant HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF.

34. A method according to claim 32 or 33 wherein the HGF is a modified form of an HGF selected from GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, EPO.

35. A method according to claim 32 or 33 wherein the modified HGF is a modified human GM-CSF having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Arg Ala Arg Arg Leu Leu Asn Leu.

36. A method according to claim 32 or 33 wherein the modified HGF is a modified human GM-CSF having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu.

37. Use of one or more modified HGFs each as defined in claim 1 or 7 in the manufacture of a medicament for the treatment of the affects of an aberrant endogenous HGF.

38. An agent comprising one or more modified HGFs each as defined in claim 1 or 7 for treating the affects of an aberrant endogenous HGF.

39. A pharmaceutical composition comprising one or more modified HGFs each as defined in claim 1 or 7 together with one or more pharmaceutically acceptable carriers and/or diluents.

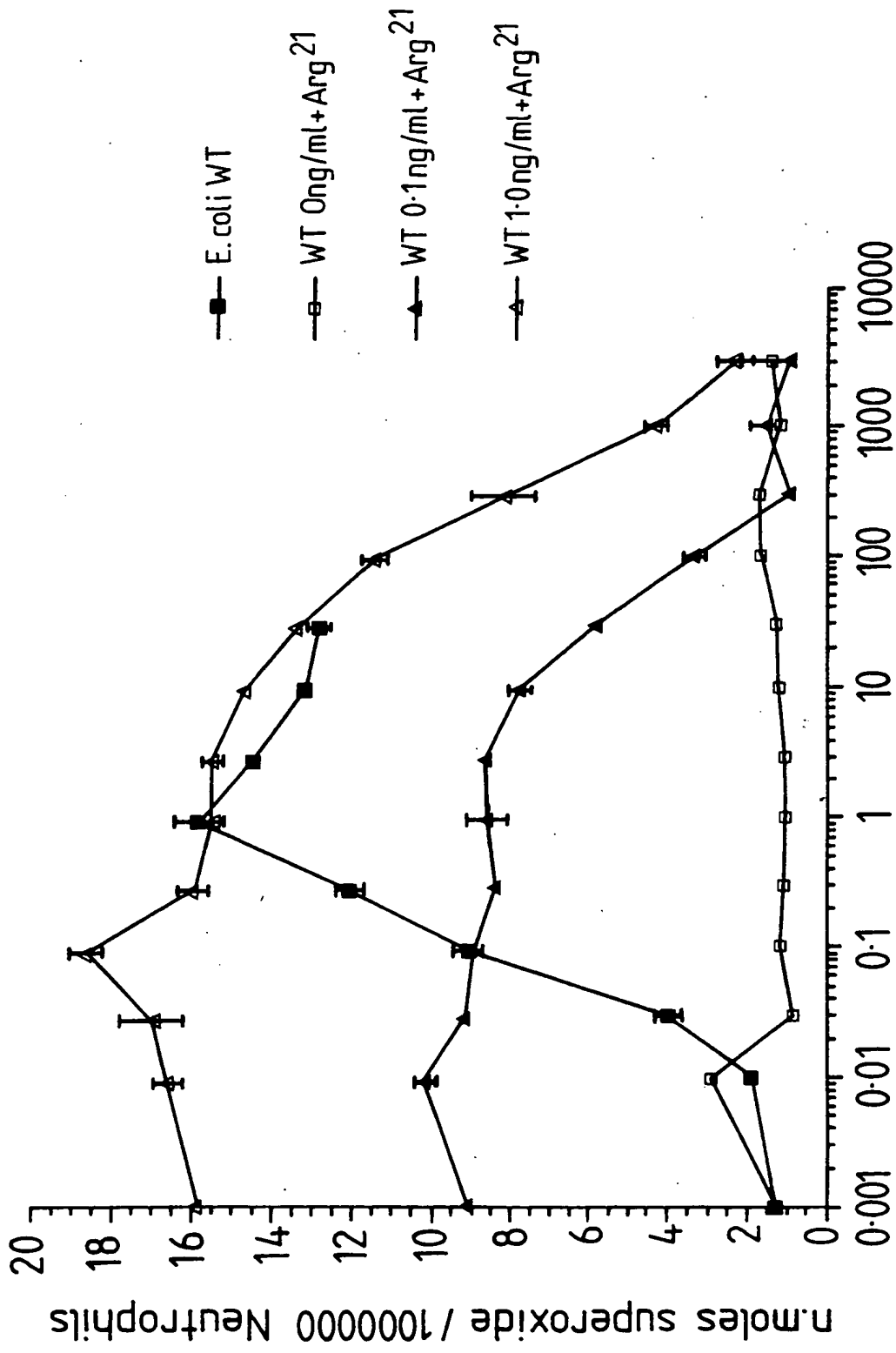

n.moles superoxide / 100000 Neutrophils
20
18
16
14
12
10
8
6
4
2
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)
E. coli WT
WT 0ng/ml+Arg21
WT 0·1ng/ml+Arg21
WT 1·0ng/ml+Arg21

FIGURE 1

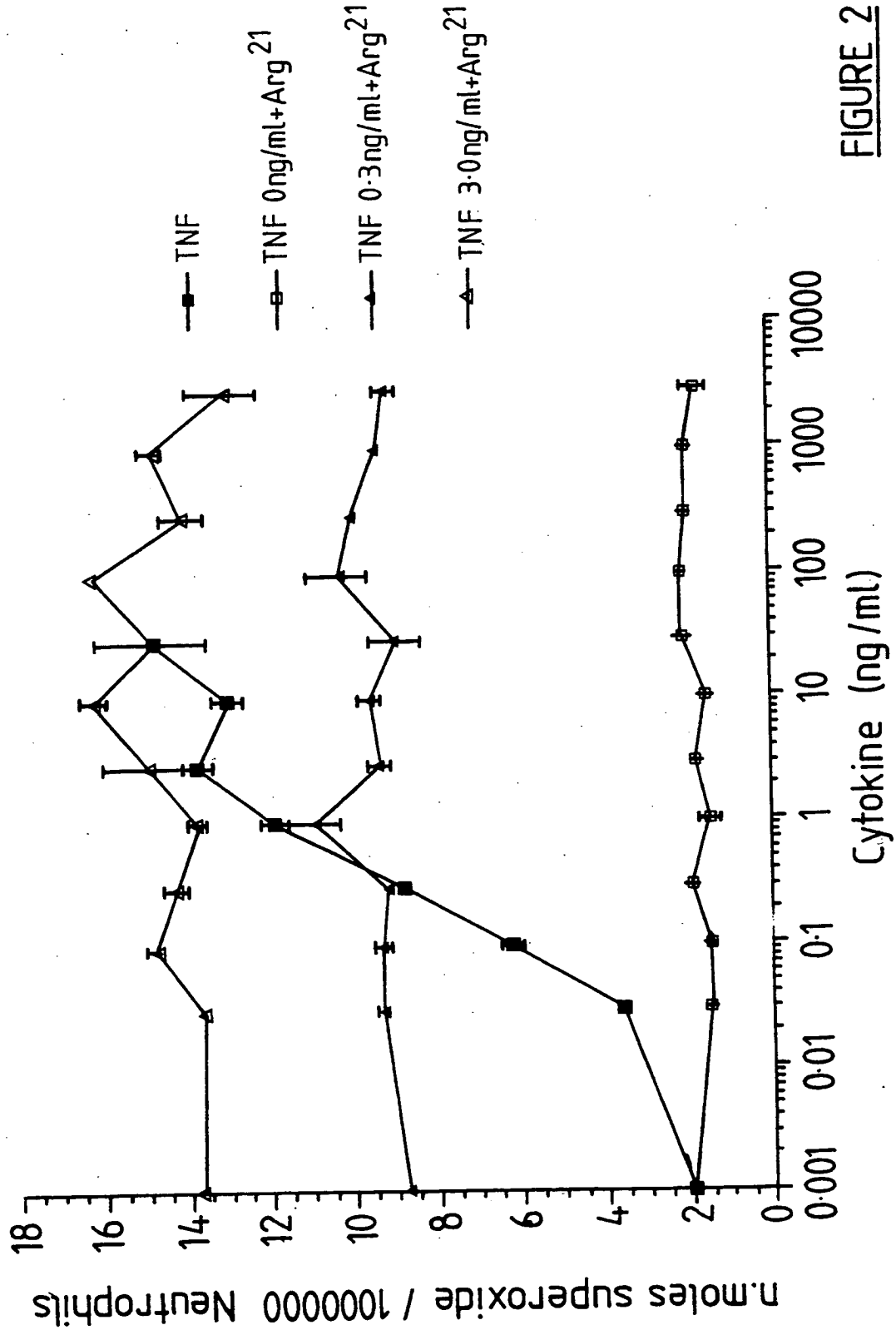
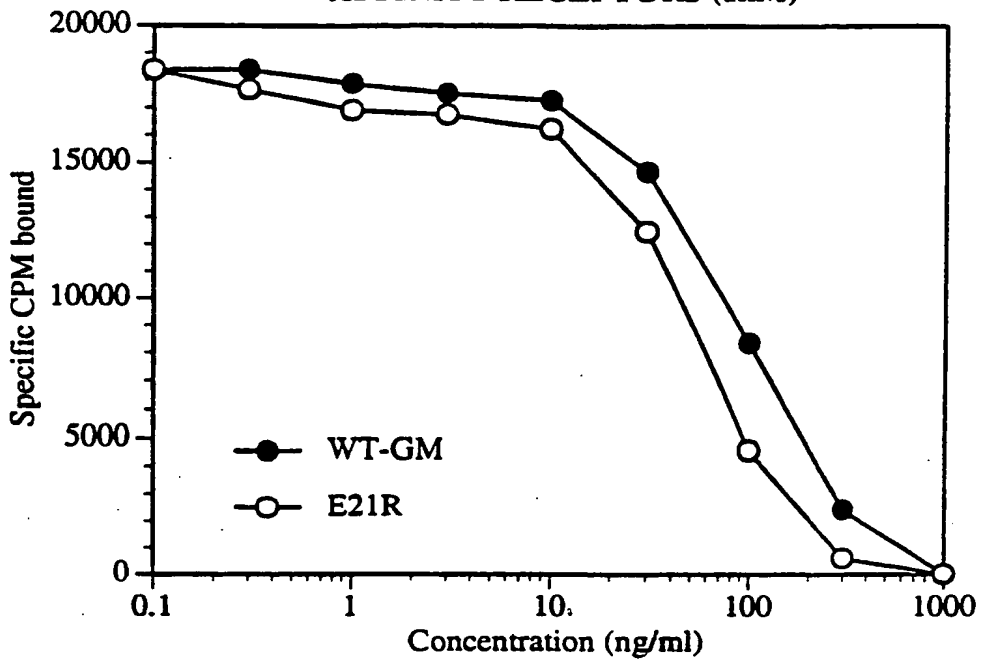

n.moles superoxide / 100000 Neutrophils
18
16
14
12
10
8
6
4
2
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)
TNF
TNF 0ng/ml+Arg21
TNF 0·3ng/ml+Arg21
TNF 3·0ng/ml+Arg21

FIGURE 2

COMPETITION OF Wt-GMCSF AND E21R FOR LOW AFFINITY RECEPTORS (1nM)



COMPETITION OF Wt-GMCSF AND E21R FOR HIGH AFFINITY RECEPTORS (100pM)

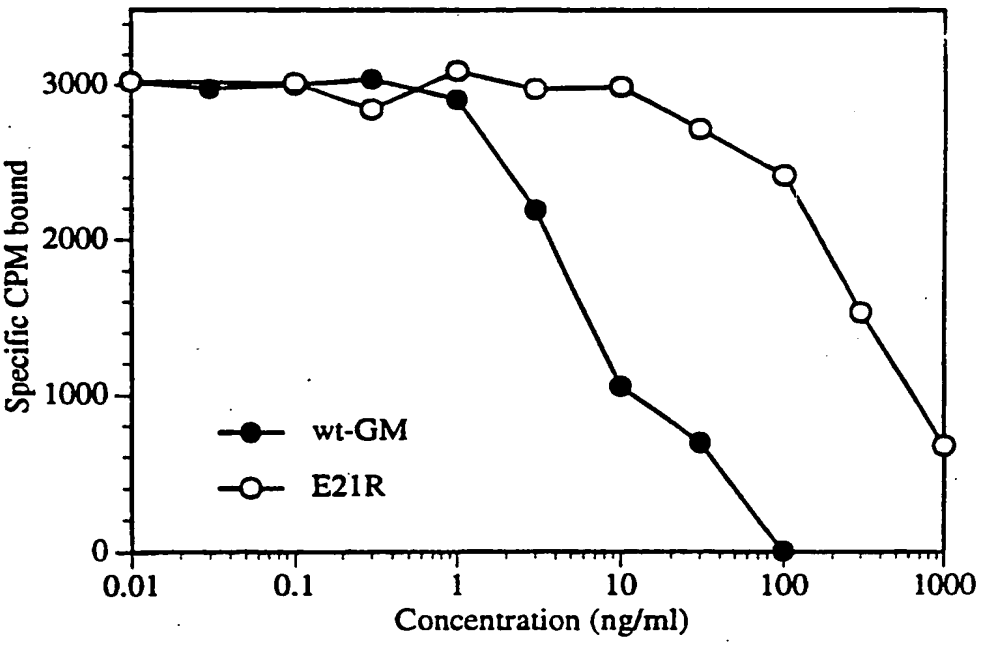


FIGURE 3

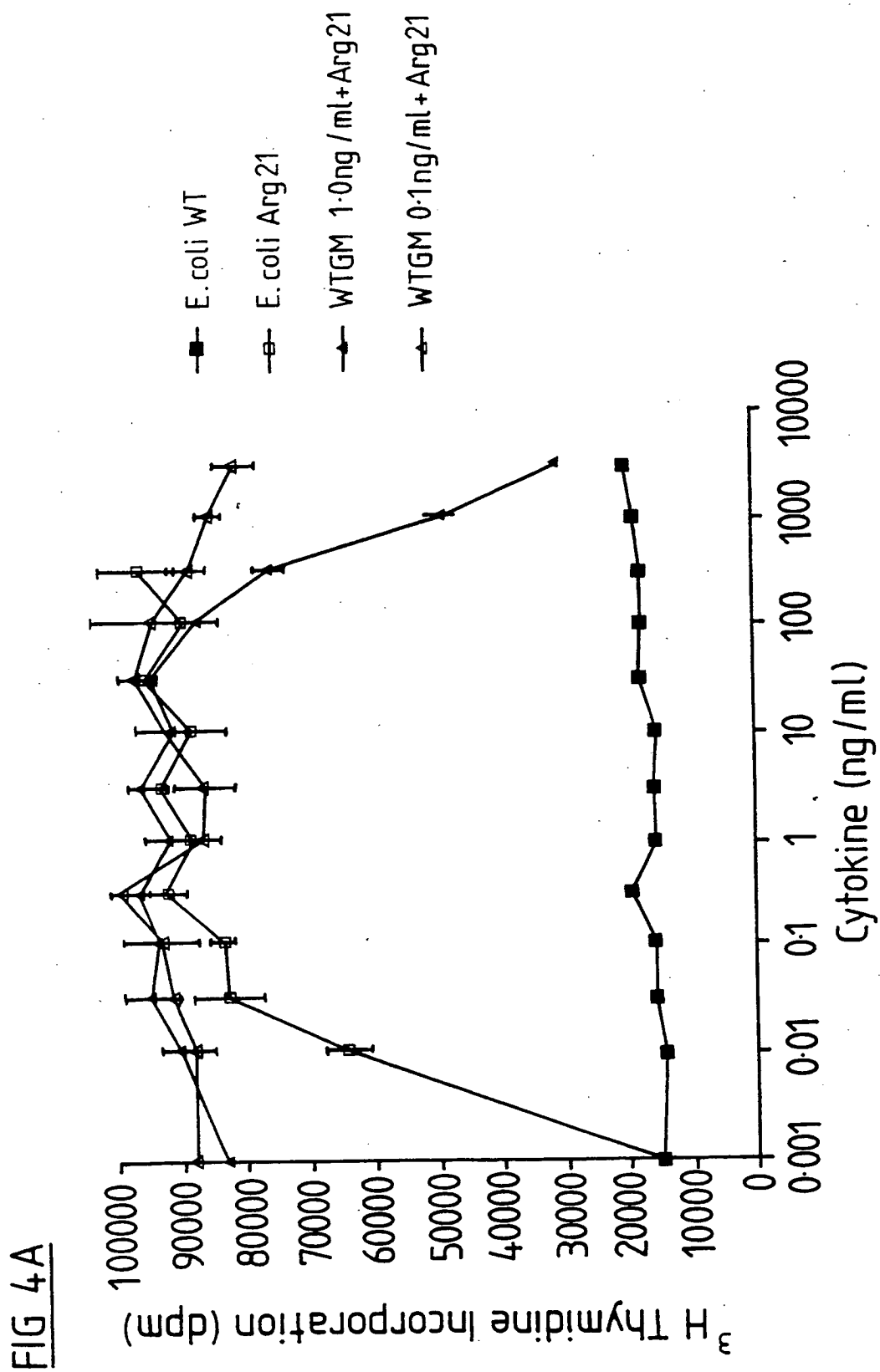

E.coli WT
E.coli Arg21
WTGM 1·0ng/ml+Arg21
WTGM 0·1ng/ml+Arg21
^{3}H Thymidine incorporation (dpm)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)

FIG 4A

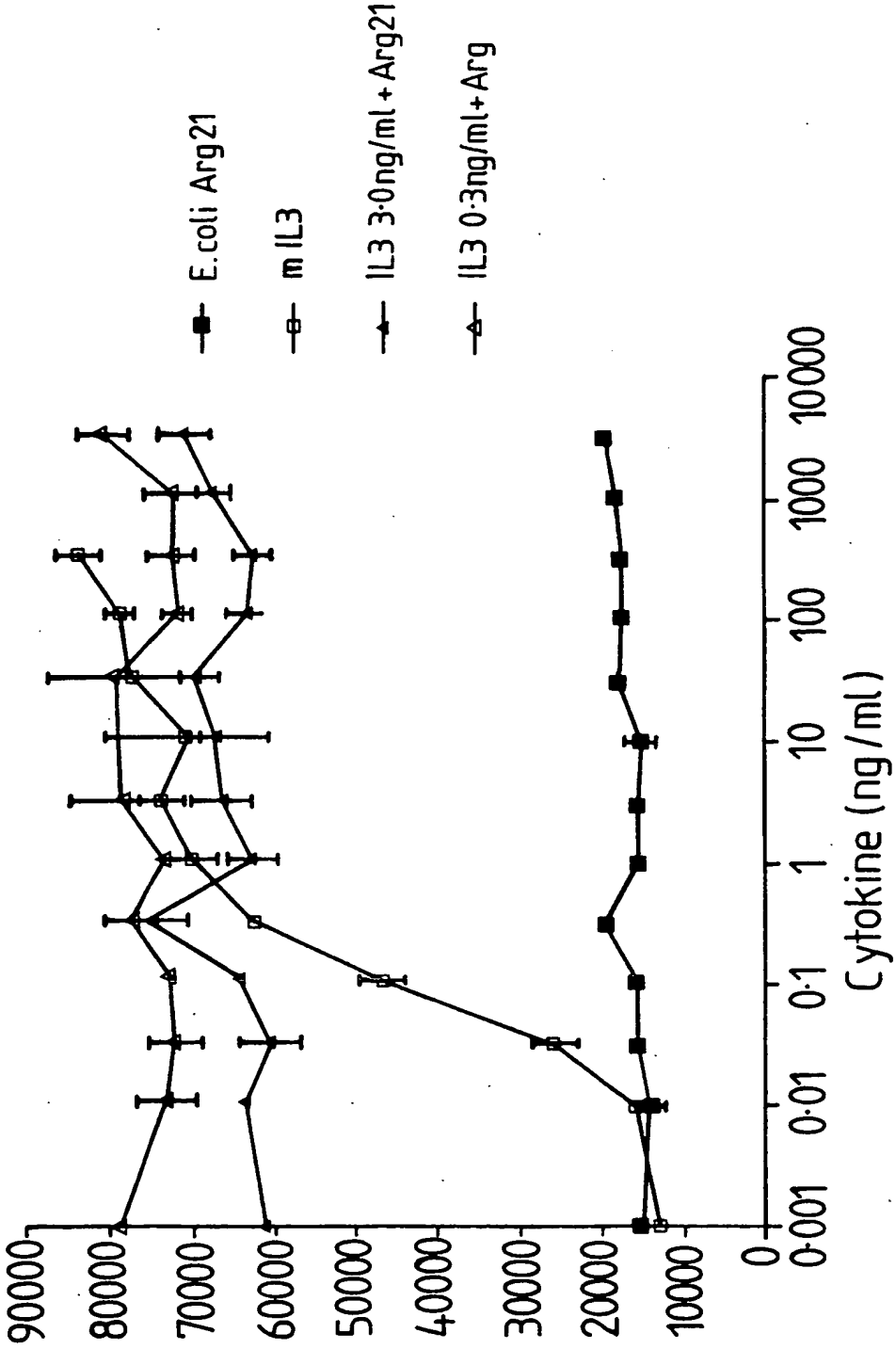

E.coli Arg21
m IL3
IL3 3·0ng/ml + Arg21
IL3 0·3ng/ml + Arg
^{3}H Thymidine incorporation (dpm)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)

FIG 4B

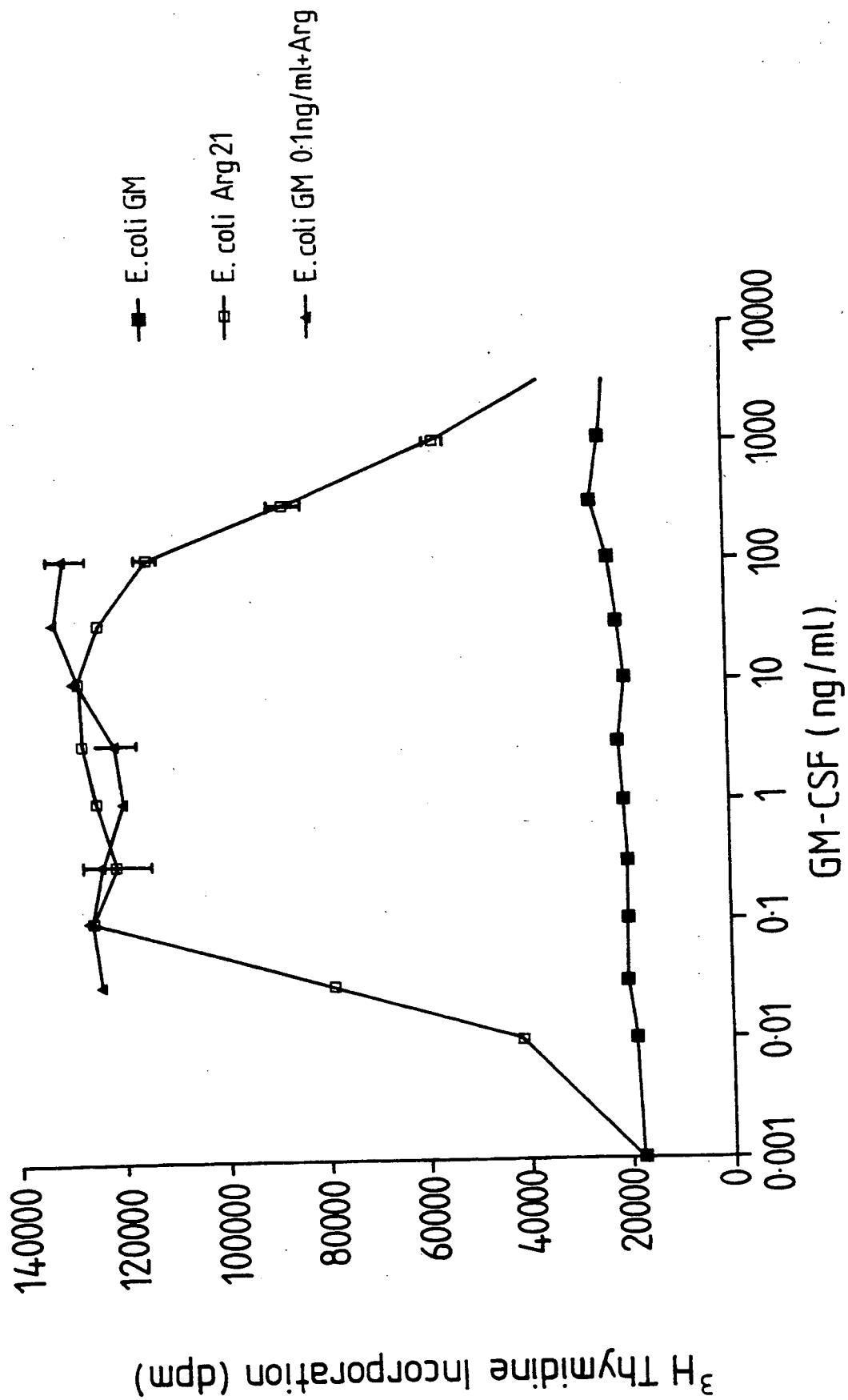

E.coli GM
E. coli Arg 21
E.coli GM 0·1ng/ml+Arg
^{3}H Thymidine Incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5A

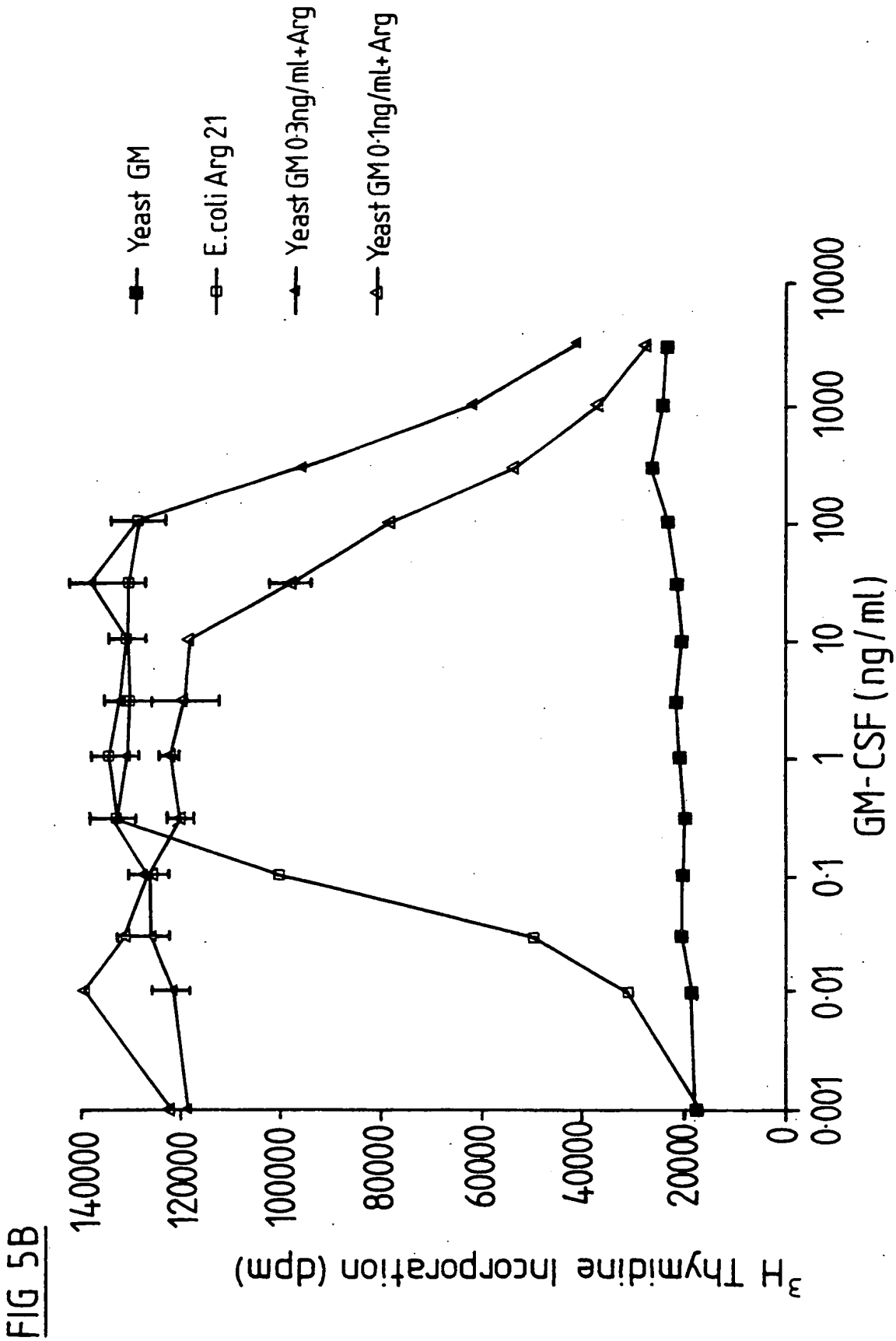

Yeast GM
E.coli Arg 21
Yeast GM 0·3ng/ml+Arg
Yeast GM 0·1ng/ml+Arg
^{3}H Thymidine Incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5B

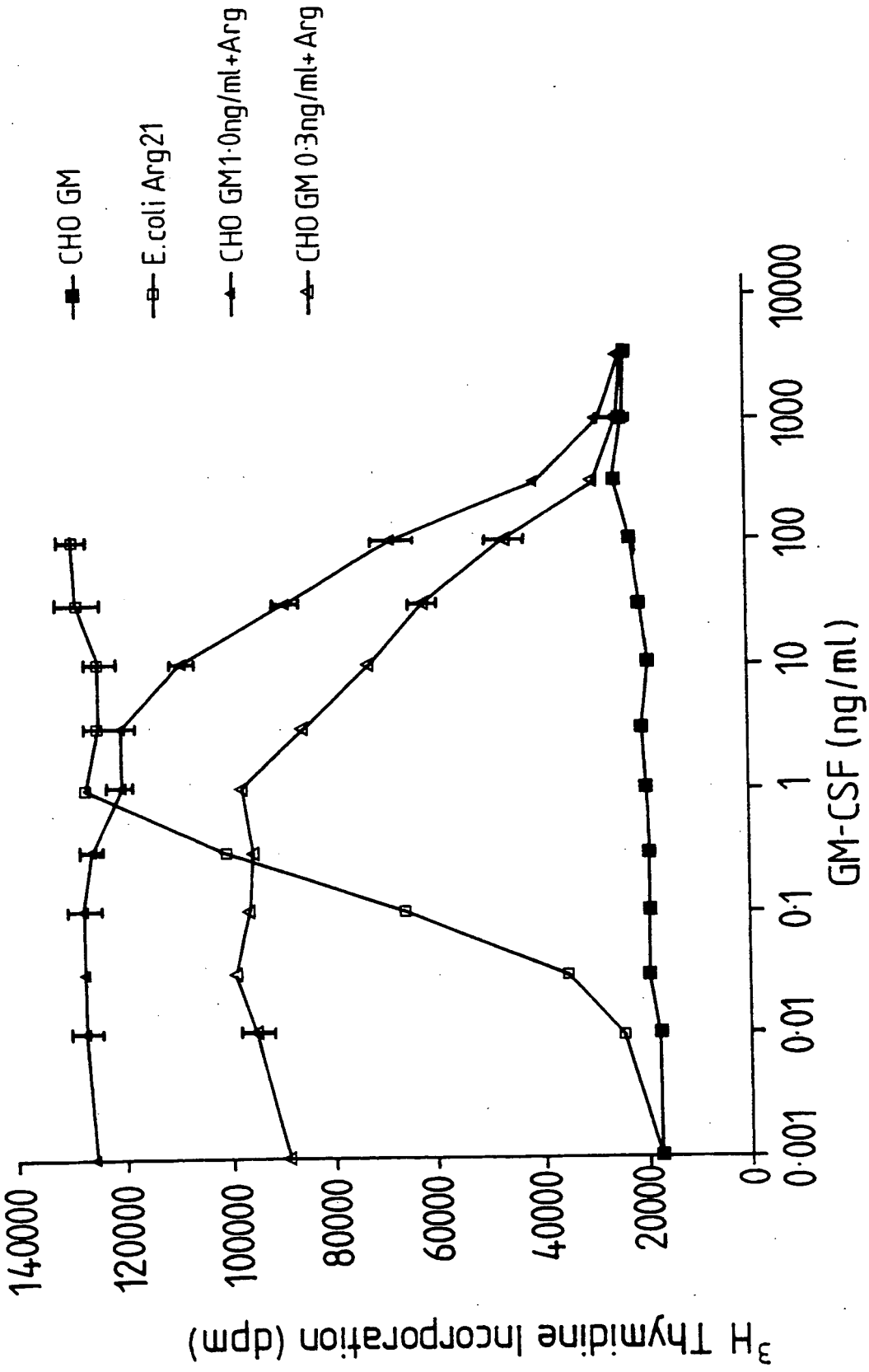

CHO GM
E.coli Arg21
CHO GM1·0ng/ml+Arg
CHO GM 0·3ng/ml+Arg
^{3}H Thymidine Incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5C

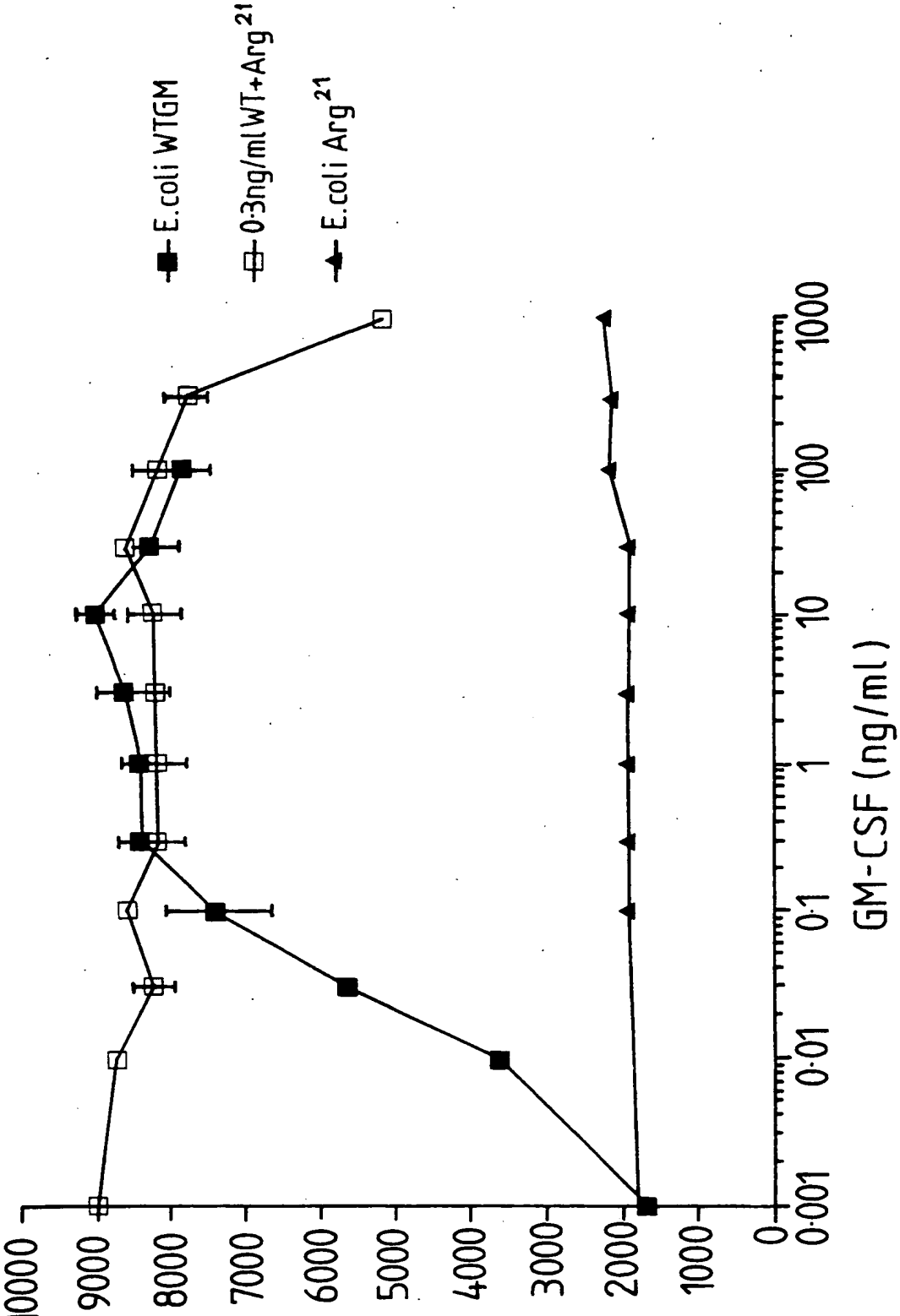

E.coli WTGM
0·3ng/ml WT+Arg21
E.coli Arg21
3H Thymidine Incorporation (dpm)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0·001
0·01
0·1
1
10
100
1000
GM-CSF (ng/ml)

FIG 6A

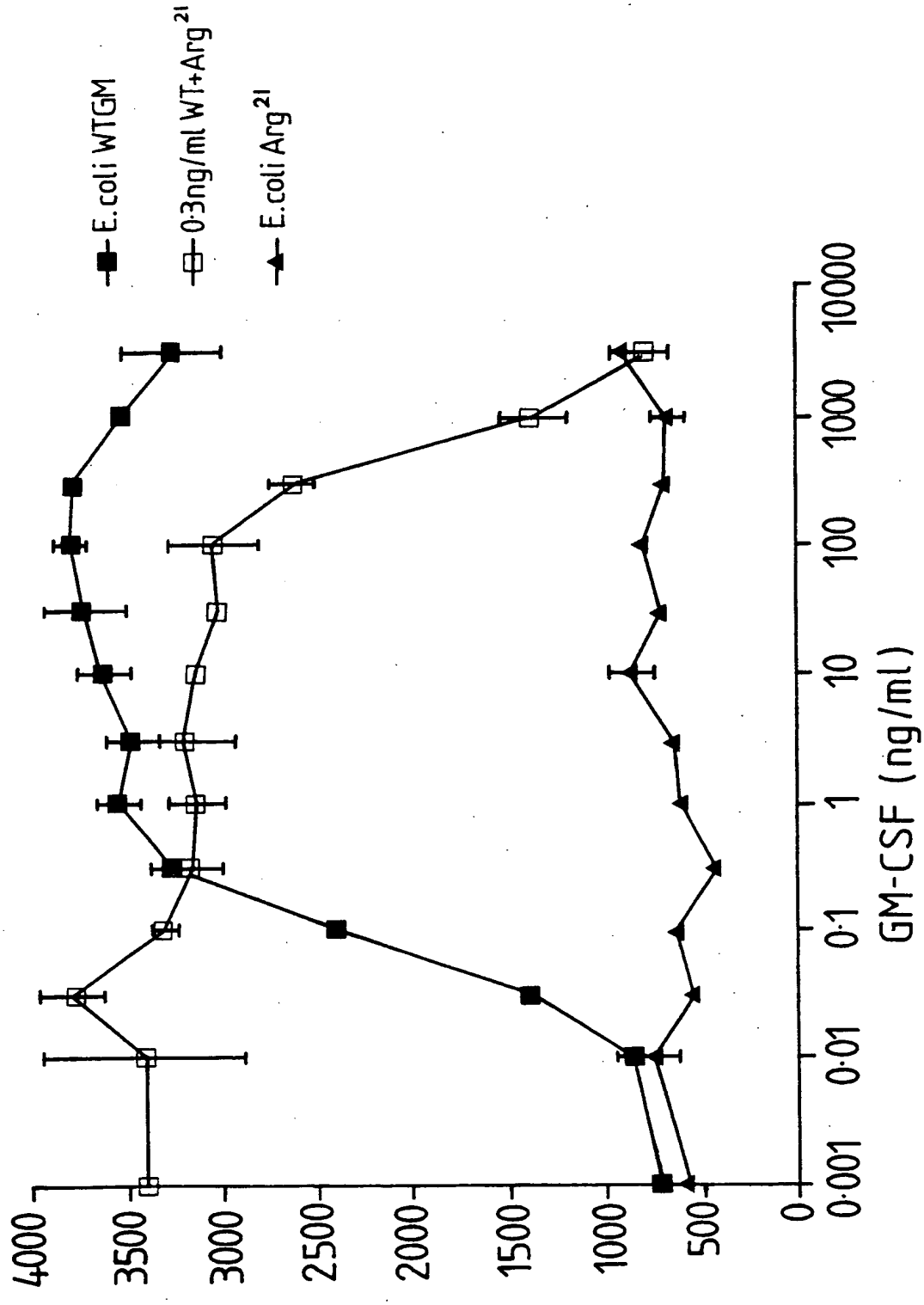

E.coli WTGM
0·3ng/ml WT+Arg21
E.coli Arg21
^{3}H Thymidine Incorporation (dpm)
4000
3500
3000
2500
2000
1500
1000
500
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 6B

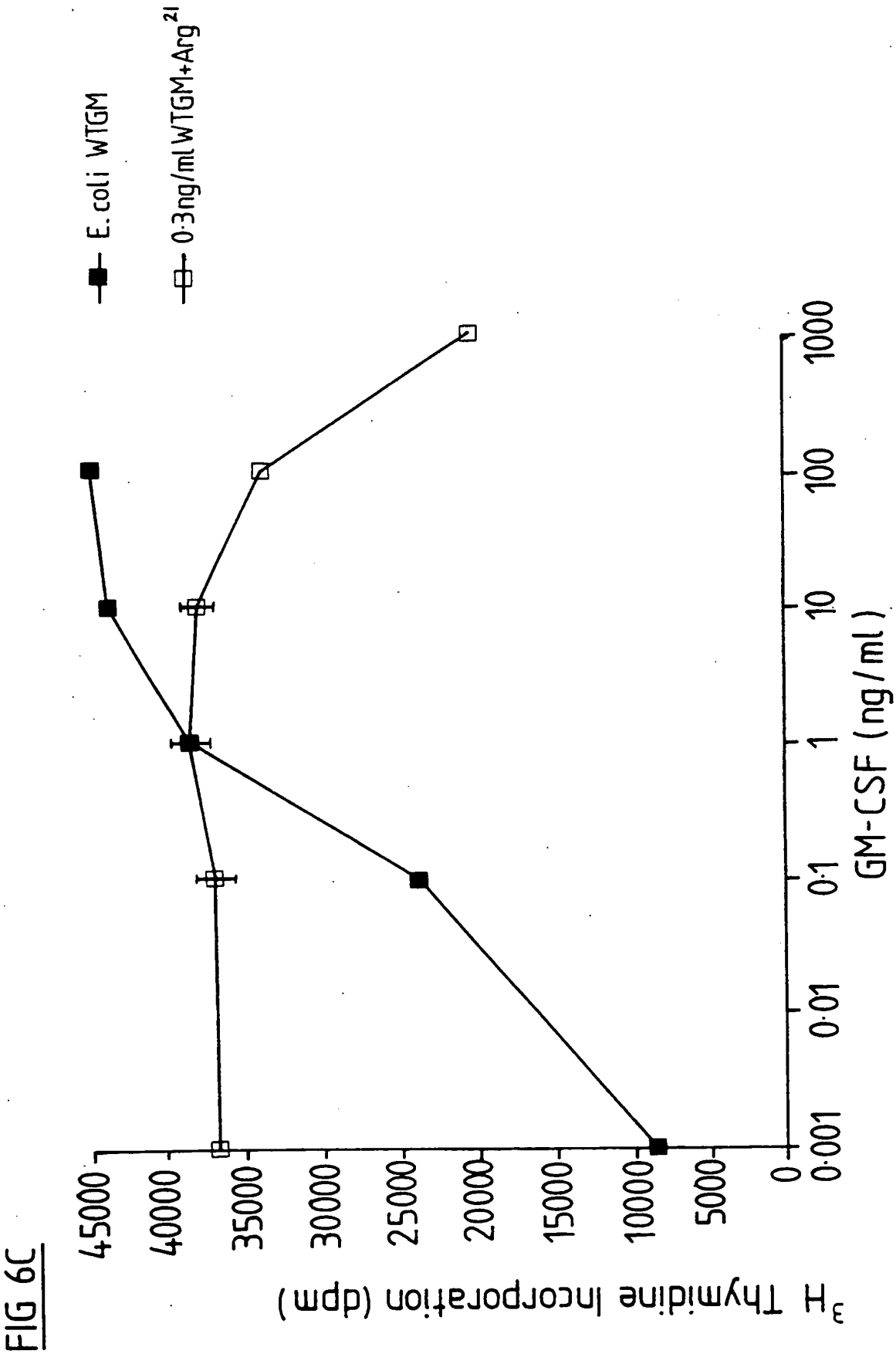

E.coli WTGM
0·3ng/ml WTGM+Arg21
3H Thymidine Incorporation (dpm)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0·001
0·01
0·1
1
10
100
1000
GM-CSF (ng/ml)

FIG 6C

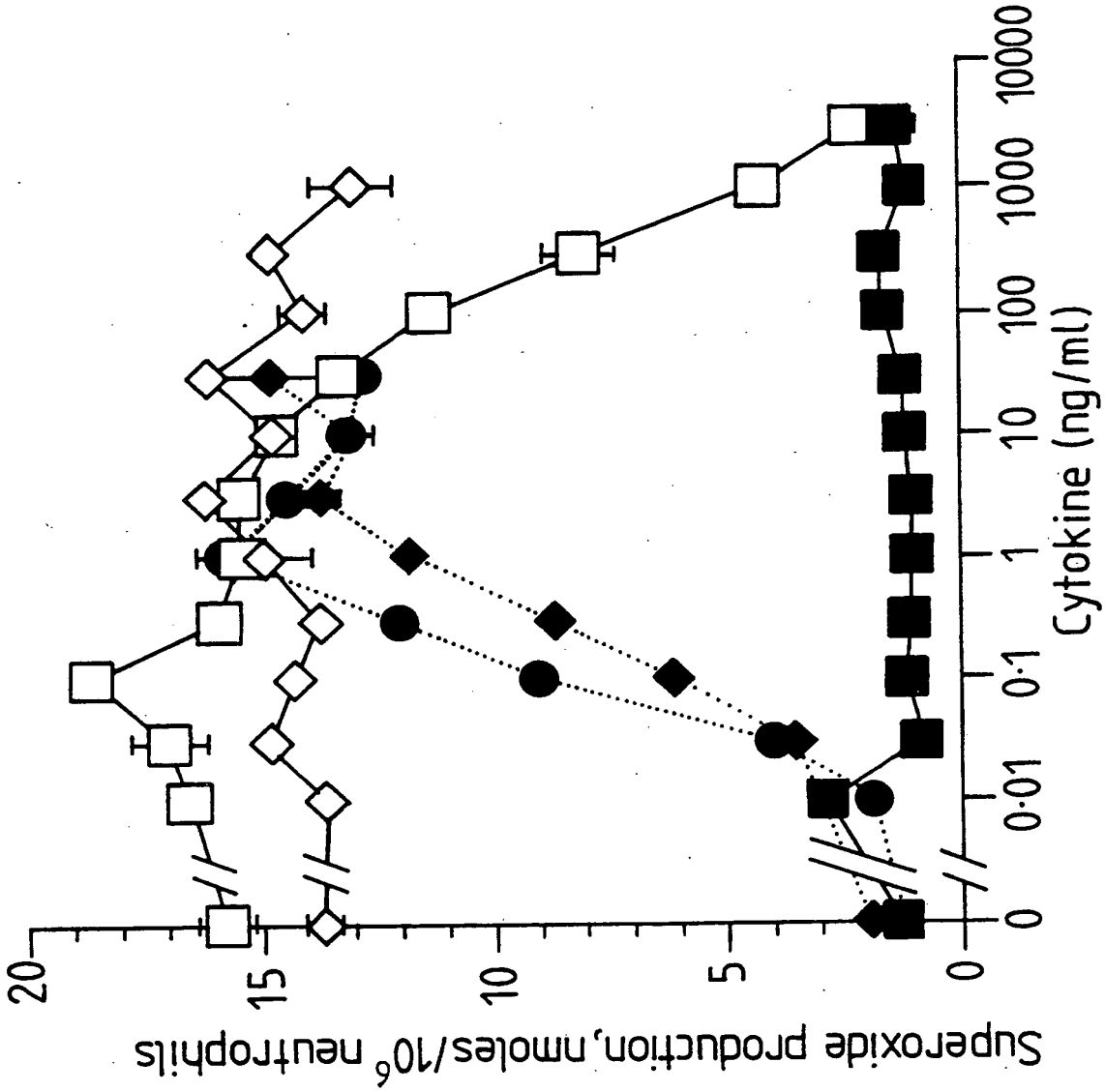

Superoxide production, nmoles/10^6 neutrophils
20
15
10
5
0
Cytokine (ng/ml)
0
0·01
0·1
1
10
100
1000
10000

FIG 7A

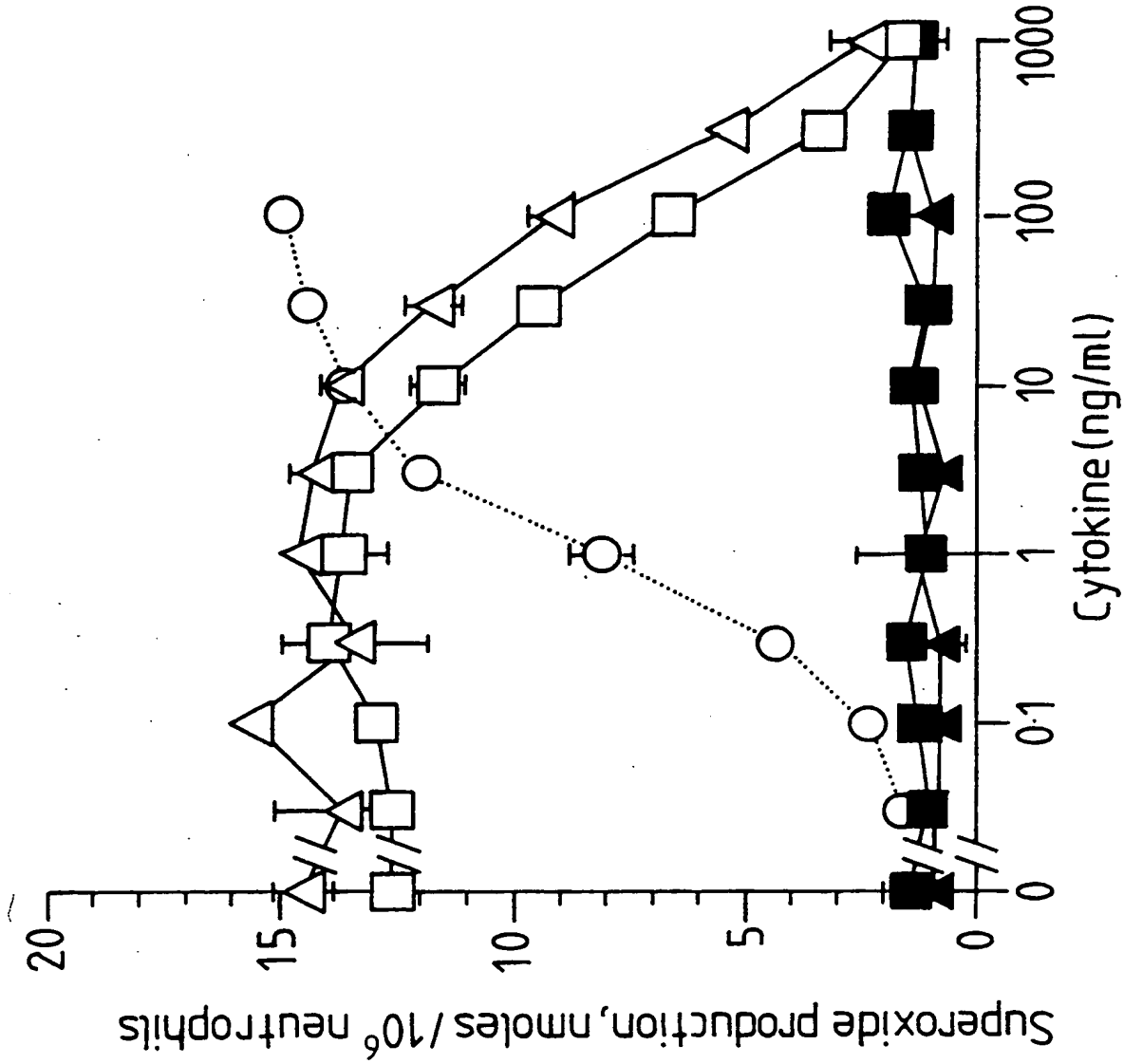

Superoxide production, nmoles / 10^6 neutrophils
20
15
10
5
0
0
0·1
1
10
100
1000
Cytokine (ng/ml)

FIG 7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00432

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[6] C07K 14/52, 14/505, 14/535, 14/54, 14/55, 14/475, C12N 15/19, 15/24, 15/26, 15/27, C12P 21/02, A61K 38/18, 38/19, 38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Derwent database; World Patent Index (WPAT); American Chemical Society database; Chemical Abstracts (CA). See "electronic data base" box below for key words used.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC: AU C12N 15/19, 15/24, 15/27, 15/26

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

WPAT, CA, DERWENT BIOTECHNOLOGY ABSTRACTS DATABASE (BIOT): (KEYWORDS) MUTEIN#; VARIANT#; ANALOG:; COLONY STIMULATING FACTOR#; INTERLEUKIN#; ERYTHROPOIETIN#; HELIC:; HELIX:

Protein sequences - see Supplemental Box

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,B,60960/90 (636641) (GIST-BROCADES N.V.) 14 February 1991 (14.02.91) pages 6, 8, 15, examples 2 and 3	1,2,4-8,15
X	AU,A,27538/92 (MEDVET SCIENCE PTY LTD) 15 April 1993 (15.04.93) page 3, figures 7 and 8	1,2,4-8,15
P,X	AU,A,56709/84 (GD SEARLE & CO.) 9 June 1994 (09.06.94) see pages 191-192, table 6	1,2,4-8,15

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1994 (15.11.94)	17 NOV 1994 (17.11.94)

Name and mailing address of the ISA/AU

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WODEN ACT 2606
AUSTRALIA

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Authorized officer

J.H. CHAN

Telephone No. (06) 2832340

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
P,X	Proceedings of National Academy of Science USA, Vol. 91, issued 21 June 1994, "Specific human granulocyte-macrophage colony-stimulating factor antagonists", (T.R. HERCUS et al), pages 5838-5842, see column 2 page 5839.	1,2,3-12
P,X	The EMBO Journal, Vol. 12, No. 13 (1993), issued 15 December 1993, "Two distinct functional sites of human interleukin-4 are identified by variants impaired in either receptor binding or receptor activation" (N. KRUSE et al) pages 5121-5129, see table 1 pages 5122-5125.	1,2,4-8,18-31
P,X	European Journal of Biochemistry, Vol. 222, issued 1 June 1994, "Neutralizing monoclonal antibodies define two different functional sites in human interleukin-4", (REUSCH, P. et al), pages 491-499, see column 1 of page 492, tables 1, 2 and 3.	1,2,4-8,18-31
X	Proceedings of National Academy of Science USA, Vol. 85, issued October 1988, "Identification of specific residues of human interleukin-2 that effect binding to the 70-KDa subunit (p70) of the interleukin-2 receptor", pages 7709-7713, see tables 1 and 2.	1,2,4-8,16,17
P,X	Biochemistry 1994, Vol. 33, issued 31 May 1994, "Mutagenic Analysis of a Receptor Contact Site on Interleukin-2: Preparation of an IL-2 Analog with Increased Potency" (BERNDT WILLIAM G. et al), pages 6571-6577, see column 2 page 6571, tables 1 and 2.	1,2,4-8,16,17
X	The EMBO Journal, Vol. 11, No. 3, issued March 1992, "Residue 21 of human granulocyte-macrophage colony stimulating factor is critical for biological activity and for high but not low affinity binding", (A.F. LOPEZ et al), pages 909-916. See column 1 of page 910 to column 2 page 912.	1,2-12
A	Biochemical and Biophysical Research Communications, Vol. 159, No. 1, 1989, issued 28 February 1989, "Mutagenesis of human granulocyte colony stimulating factor", pages 103-111.	
A	Proceedings of National Academy of Science, Vol. 89, issued 1 December 1992, "A human interleukin-3 analog with increased biological and binding activities", (T. KUGO et al), pages 11842-11846.	
A	Biochimica et Biophysica Acta, Vol. 1041 (1990), "Theoretical conformational analysis of a family of alpha-helical immunocytokines (V.P. ZAV'YALOV et al), pages 178-185.	

B. FIELDS SEARCHED (supplemental box)

The following protein subsequences were searched in CA database in STN International:

HVNAIQ[HKR]ARRLLNL
ALVK[HKR]TLALLSTHRTL
NMI[HKR][DE]IITHL
NMI[DE][HKR]IITHL
NMI[HKR][HKR]IITHL
LLL[HKR]LQMIL
ITLQ[HKR]IIKTL
AYIL[HKR]GISALRK
GDQY[HKR]SVLMVSI
AGIL[HKR]INFLINKMQGD
NMLR[HKR]LADAFS
FLLKCL[HKR]QVRKI
YLLEAK[HKR]AENITTG

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	60690/90	US	5331148	WO	91/03029		
AU	27538/92	EP	609280	WO	93/07171		
AU	56709/94	AU	56125/94	WO	94/12638	WO	94/12639

END OF ANNEX